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EURL for Fungi and Oomycetes

**Funded by
the European Union**

Towards a more reliable detection of quarantine *Elsinoe* species on citrus: Beyond sequences, primers and probes

Pedro-Pablo Parra Giraldo, Jaime Aguayo , Camille Renault, Marion Petit-Garcia and Renaud Ios
EURL for Fungi and Oomycetes

Vienna, April 2026



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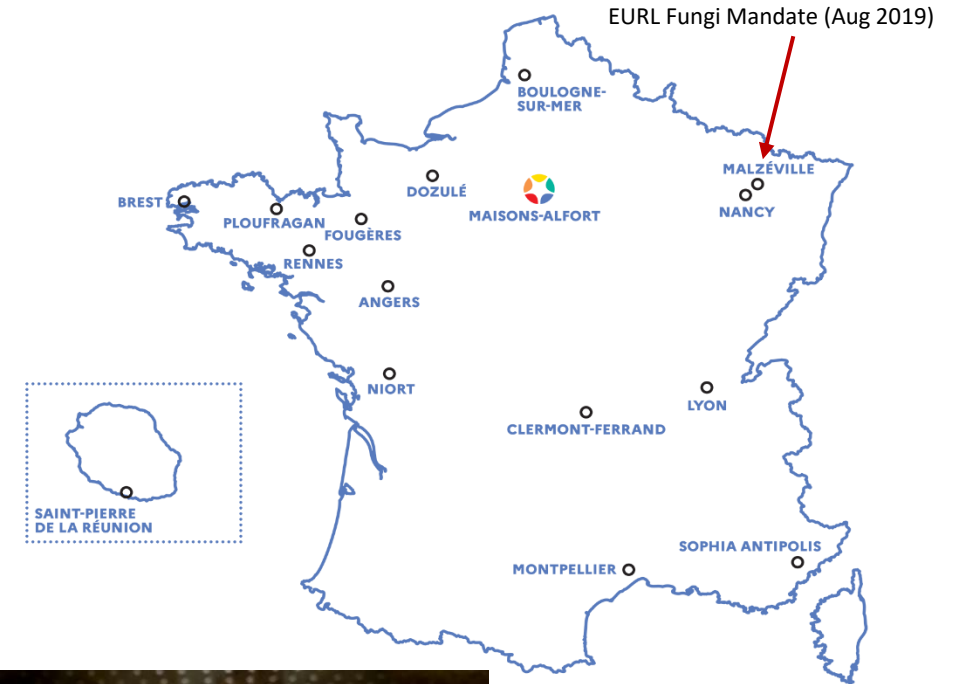


The French Agency for Food, Environmental and Occupational Health & Safety (ANSES)



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STANISLAS SQUARE



THE DAUM GLASSWORKS

© Collection Daum, Nancy's Fine Arts Museum

Mycology Unit of the ANSES Plant Health laboratory



The ANSES Plant Health Laboratory (**LSV**) is structured in 6 local units, specialized by field in plant pathology (90 people)

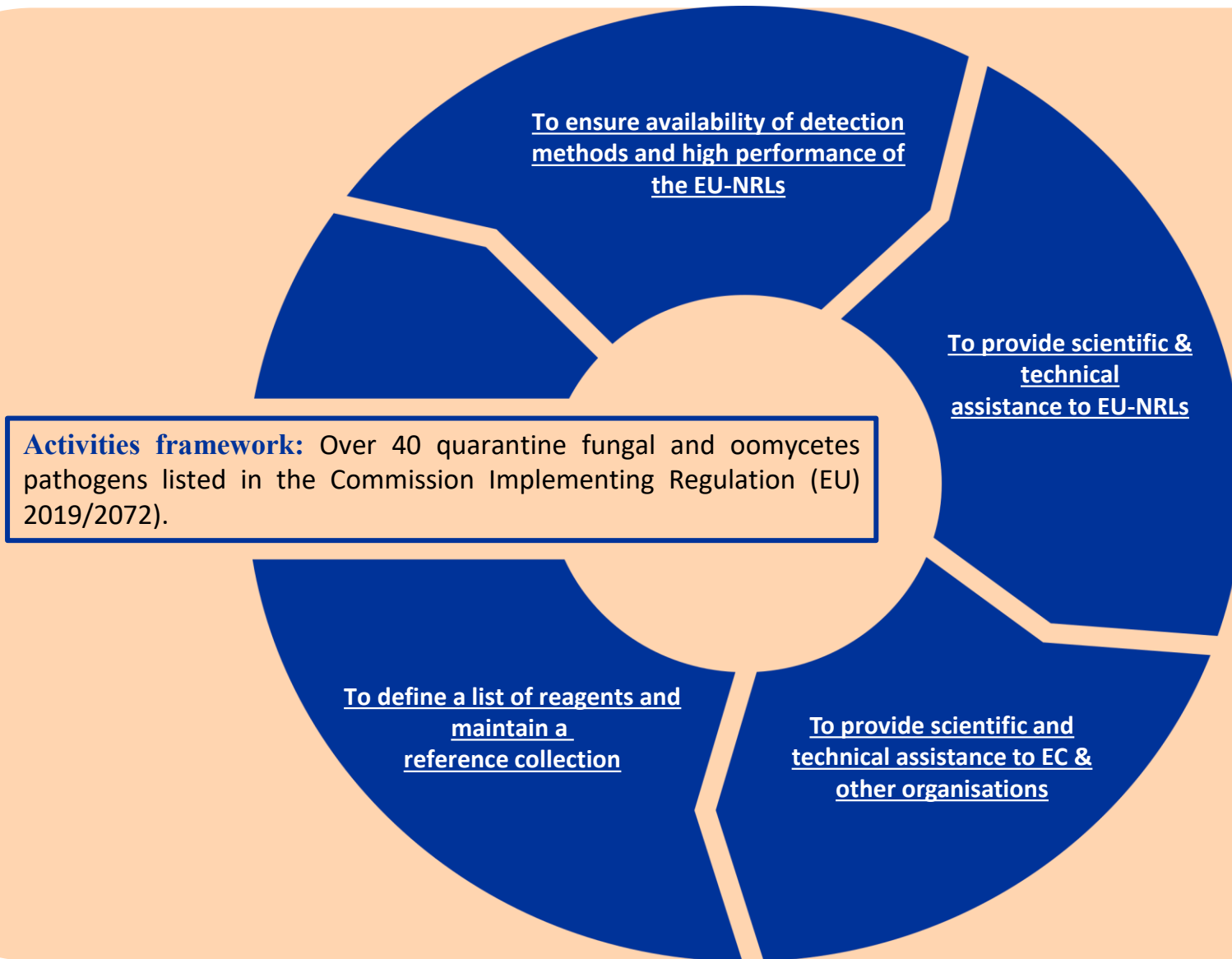


LSV Mycology Unit (14 people)

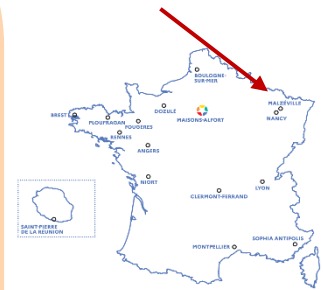
French NRL

EURL Fungi (Mandate)

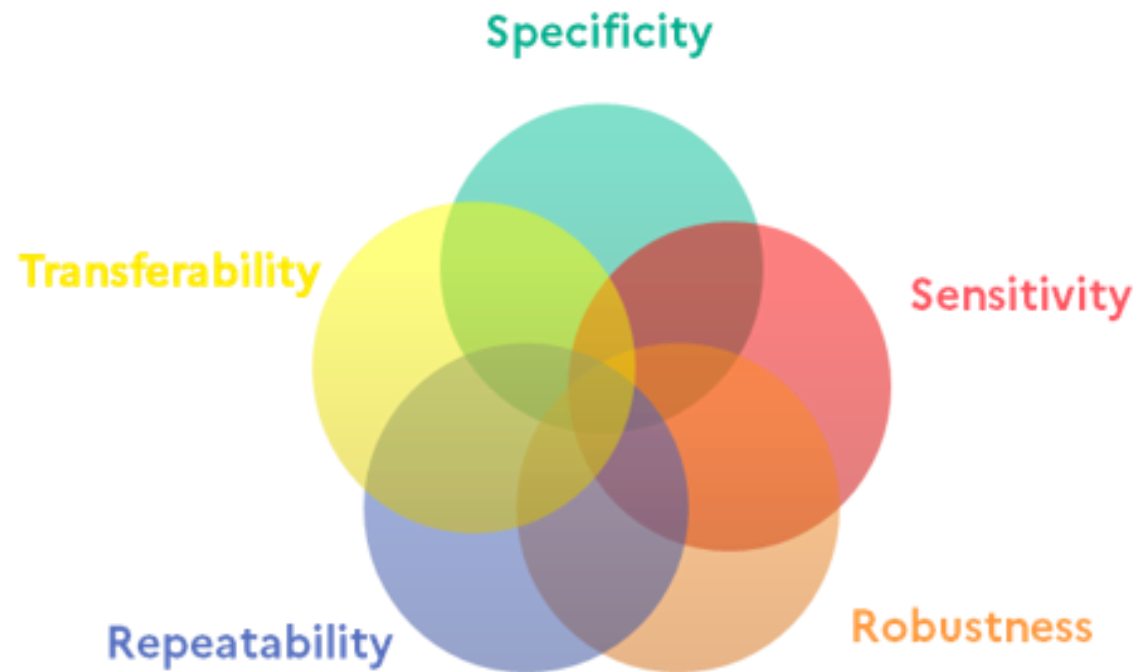
Our EURL mandate:



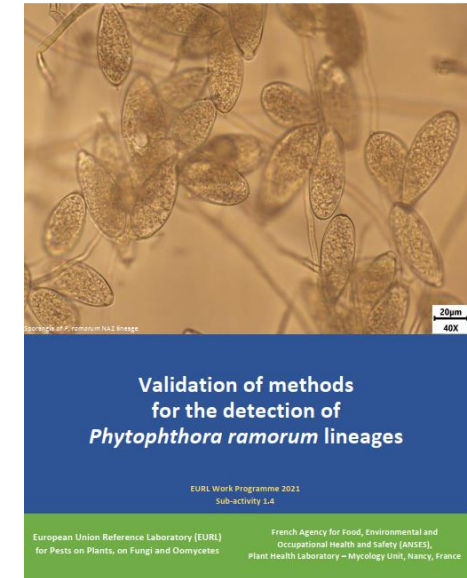
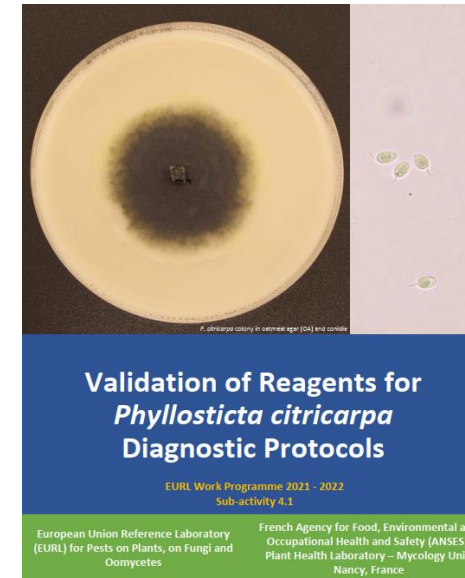
EURL Mandate (Aug 2019)



To ensure availability of detection methods



Goal: To provide reliable detection methods so the EU-NRLs adopt them for official analysis.



List of Regulated Quarantine Pests: Fungi and oomycetes



B. Fungi and oomycetes	
1.	<i>Anisogramma anomala</i> (Peck) E. Müller [CRSPAN]
2.	<i>Apiosporina morbosus</i> (Schwein.) Arx [DIBOMO]
3.	<i>Atropellis</i> spp. [IATRPG]
4.	<i>Botryosphaeria kuwatsukai</i> (Hara) G.Y. Sun and E. Tanaka [PHYOPI]
5.	<i>Bretziella fagacearum</i> (Bretz) Z.W. de Beer, T.A. Duong & M.J. Wingfield, comb. nov. [CERAFSA]
6.	<i>Chrysomyxa arctostaphyli</i> Dietel [CHMYAR]
7.	<i>Cronartium</i> spp. [ICRONG], except <i>Cronartium gentianeum</i> , <i>Cronartium pini</i> (Willdenow) Jørstad [ENDCPI] and <i>Cronartium ribicola</i> Fischer [CRONRI].
8.	<i>Davidsoniella virescens</i> (R.W. Davidson) Z.W. de Beer, T.A. Duong & M.J. Wingfield [CERAVI]
9.	<i>Elsinoë australis</i> Bitanc. & Jenkins [ELSIAU]
10.	<i>Elsinoë citricola</i> X.L. Fan, R.W. Barreto & Crous [ELSICI]
11.	<i>Elsinoë fawcettii</i> Bitanc. & Jenkins [ELSIFA]
12.	<i>Fusarium oxysporum</i> f. sp. <i>albedinis</i> (Kill. & Maire) W.L. Gordon [FUSAAL]
13.	<i>Guignardia loricata</i> (Sawada) W. Yamam. & Kaz. Itô [GUIGLA]
14.	<i>Gymnosporangium</i> spp. [IGYMNG], except:

15.	<i>Coniferiporia sulphurascens</i> (Pilát) L.W. Zhou & Y.C. Dai [PHELSU]
16.	<i>Coniferiporia weirii</i> (Murrill) L.W. Zhou & Y.C. Dai [INONWE]
17.	<i>Melampsora farlowii</i> (Arthur) Davis [MELMFA]
18.	<i>Melampsora medusae</i> f. sp. <i>tremuloides</i> Ghain [MELMMT]
19.	<i>Mycodiella laticis-leptolepidis</i> (Kaz. Itô, K. Satô & M. Ota) Crous [MYCOLL]
20.	<i>Phoma andina</i> Turkensteen [PHOMAN]
21.	<i>Phyllosticta citricarpa</i> (McAlpine) Van der Aa [GUIGCI]
22.	<i>Phyllosticta solitaria</i> Ellis & Everhart [PHYSSL]
23.	<i>Phymatotrichopsis omnivora</i> (Duggar) Hennebert [PHMPOM]
24.	<i>Phytophthora ramorum</i> (non-EU isolates) Werres, De Cock & Man in 't Veld [PHYTRA]
25.	<i>Pseudocercospora angolensis</i> (T. Carvalho & O. Mendes) Crous & U. Braun [CERCAN]
26.	<i>Pseudocercospora pini-densiflorae</i> (Hori & Nambu) Deighton [CERSPD]
27.	<i>Puccinia pittieriana</i> Hennings [PUCCPT]
28.	<i>Septoria malagutii</i> E.T. Cline [SEPTLM]
29.	<i>Sphaerulina musiva</i> (Peck) Quaedvl. Verkley & Crous. [MYCOPP]
30.	<i>Stegophora ulmea</i> (Fr.) Syd. & P. Syd [GNOMUL]

31.	<i>Thecaphora solani</i> Thirumalachar & O'Brien) Mordue [THPHSO]
32.	<i>Tilletia indica</i> Mitra [NEOVIN]
33.	<i>Venturia nashicola</i> S. Tanaka & S. Yamamoto [VENTNA]
1.	<i>Ceratocystis platani</i> (J. M. Walter) Engelbr. & T. C. Harr [CERAFP]
2.	<i>Fusarium circinatum</i> Nirenberg & O'Donnell [GIBBCI]
3.	<i>Geosmithia morbida</i> Kolarik, Freeland, Uteley & Tisserat [GEOHMO]
4.	<i>Synchytrium endobioticum</i> (Schilb.) Percival [SYNCEN]

> 40 taxa of fungi and oomycetes are listed as regulated quarantine organisms (QO)

There are three *Elsinoe* species regulated in the EU

An overview of *Elsinoe* spp.

High number of species and hosts associations

PHYLOGENY AND TAXONOMY OF GENUS *ELSINOË*

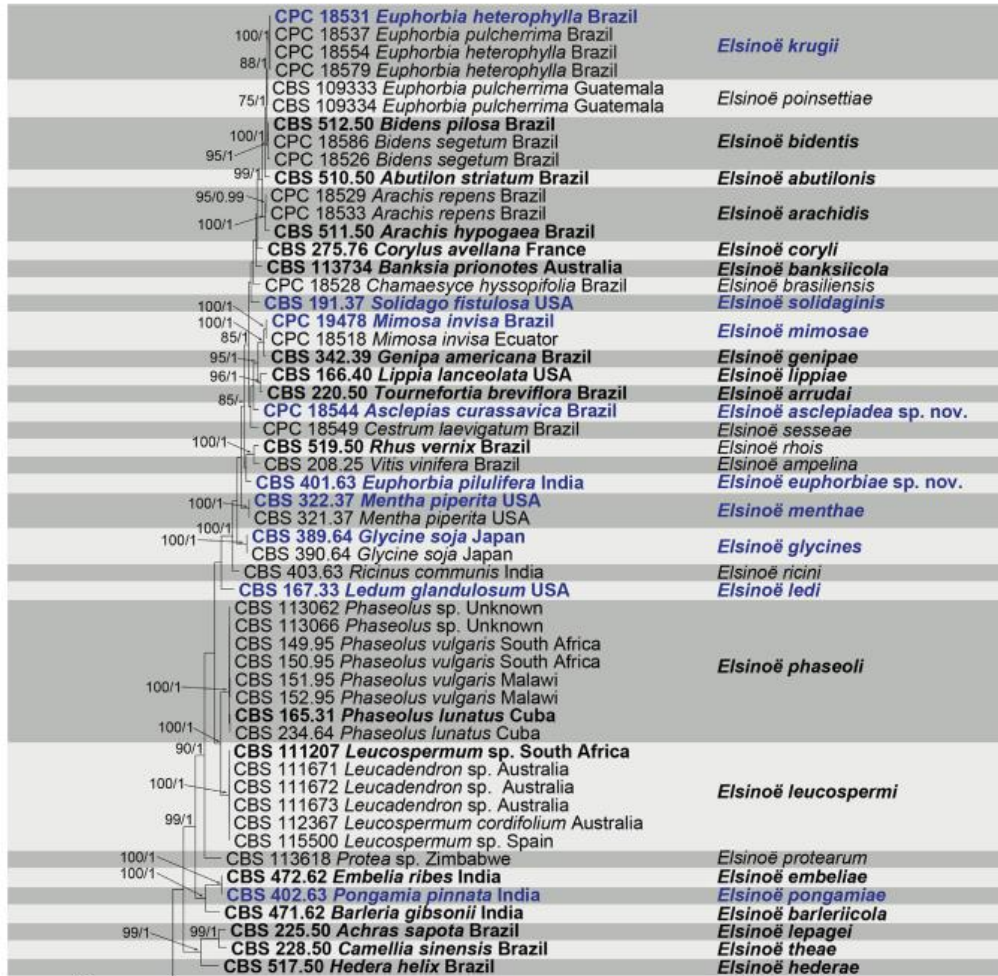
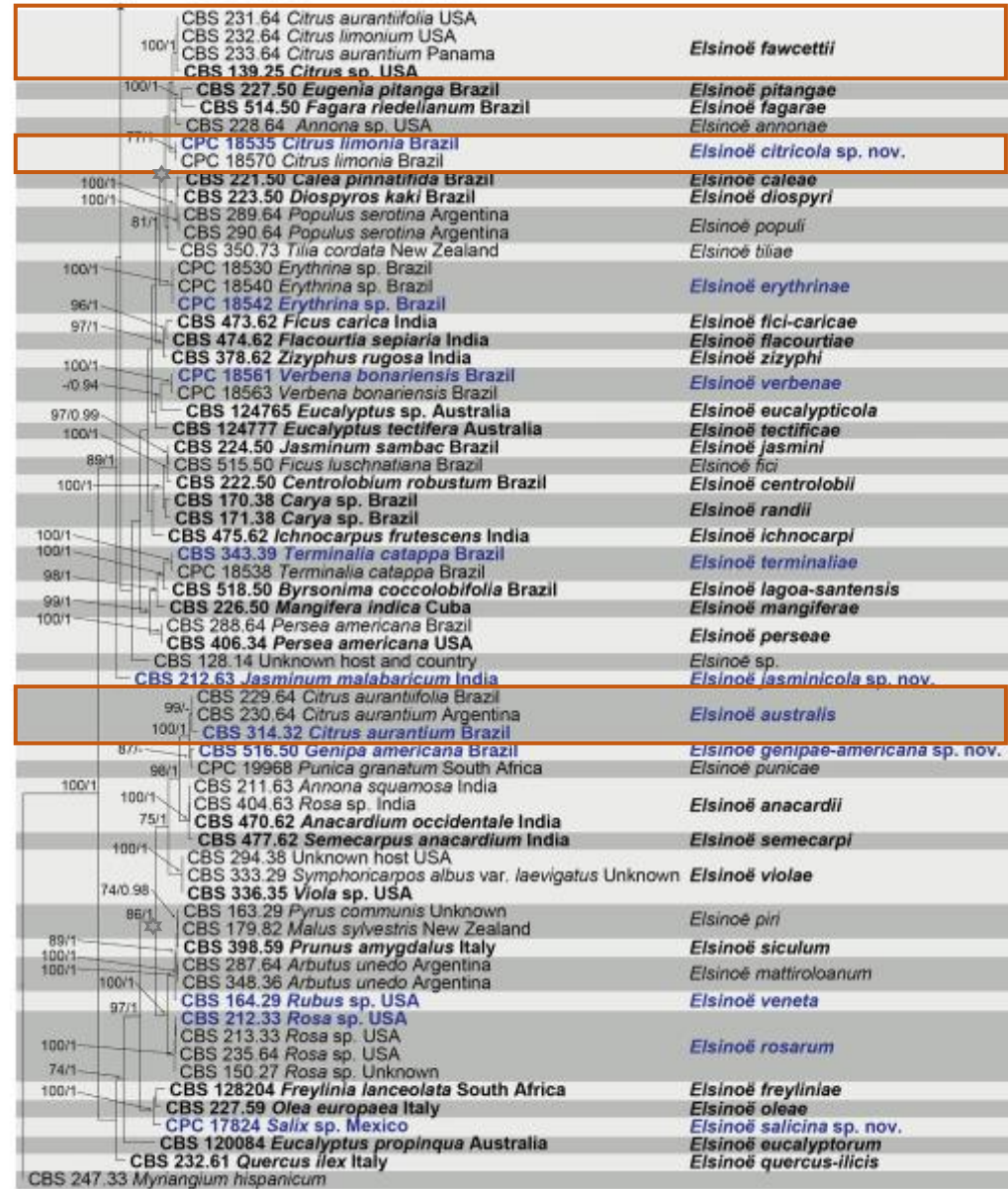


Fig. 4. Maximum parsimony (MP) phylogram of *Elsinoë* based on a combined matrix of ITS, LSU, *rpb2* and *TEF1-α* genes. MP bootstrap support values above 73 % and posterior probabilities above 0.93 from BI are shown at the first and second positions at the nodes. The scale bar represents 90 nucleotide changes. Ex-type strains are in bold. New species and ex-epitypes are in blue.

FAN ET AL.



ITS, LSU, RPB2, TEF1

Fan et al. 2017



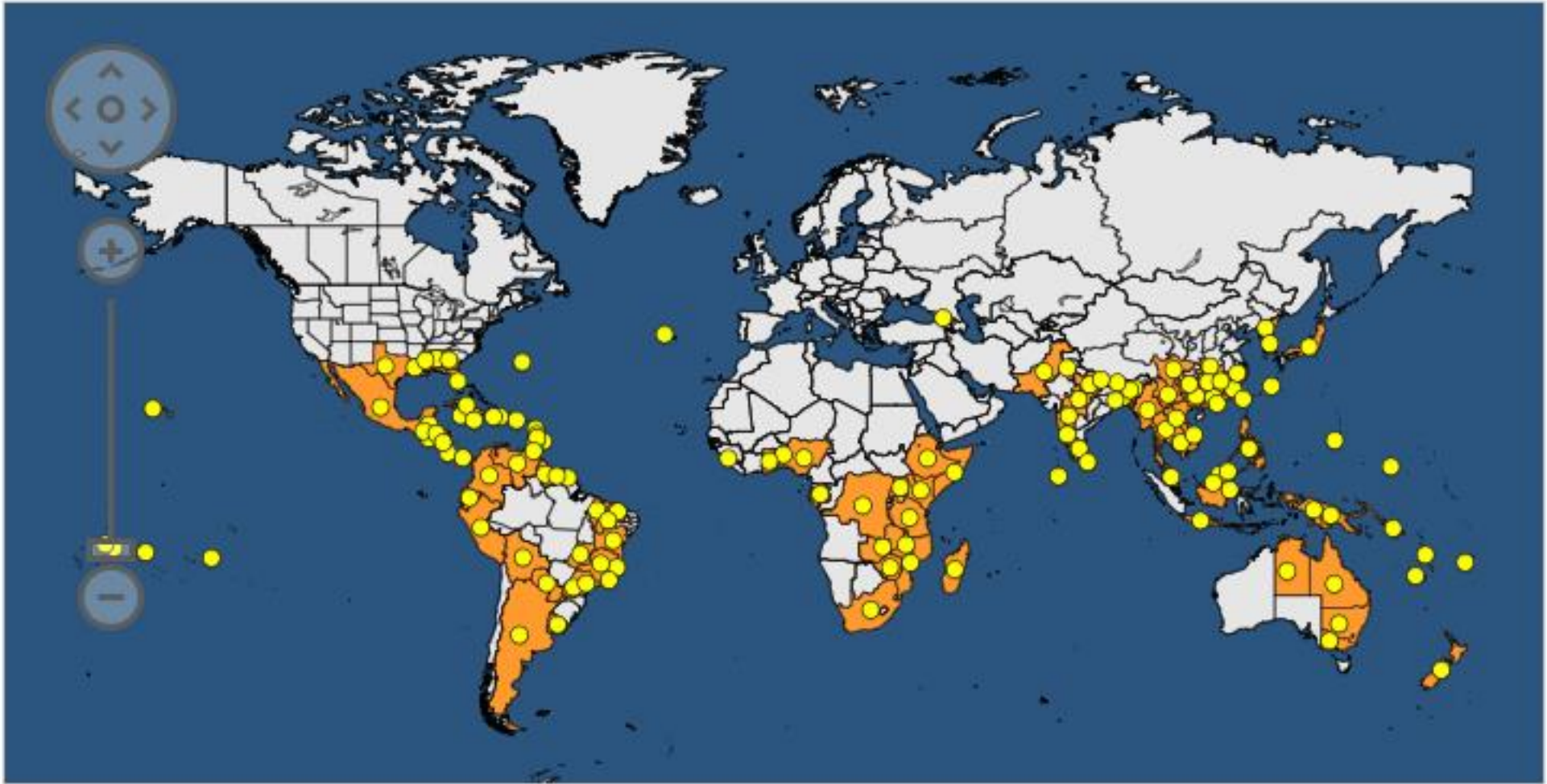
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Considerations on EU-regulated *Elsinoe* species

- They impact the plant vigor; yield and the aspect of the fruit (**scab symptoms**)
- Only three species were known to occur on Citrus = *E. australis*, *E. citricola* and *E. fawcettii*
- **Difficult to isolate** and **variable macromorphological characteristics** (e.g., *E. fawcettii*, *E. australis*)
- Identification had/has been traditionally done using one or two genes/loci (ITS, TEF1) (**enough?**).
 - ✓ Underestimation of their distribution, diversity, co-occurrence, etc.
- Multiple **pathotypes** have been reported in the past (**different species?**)



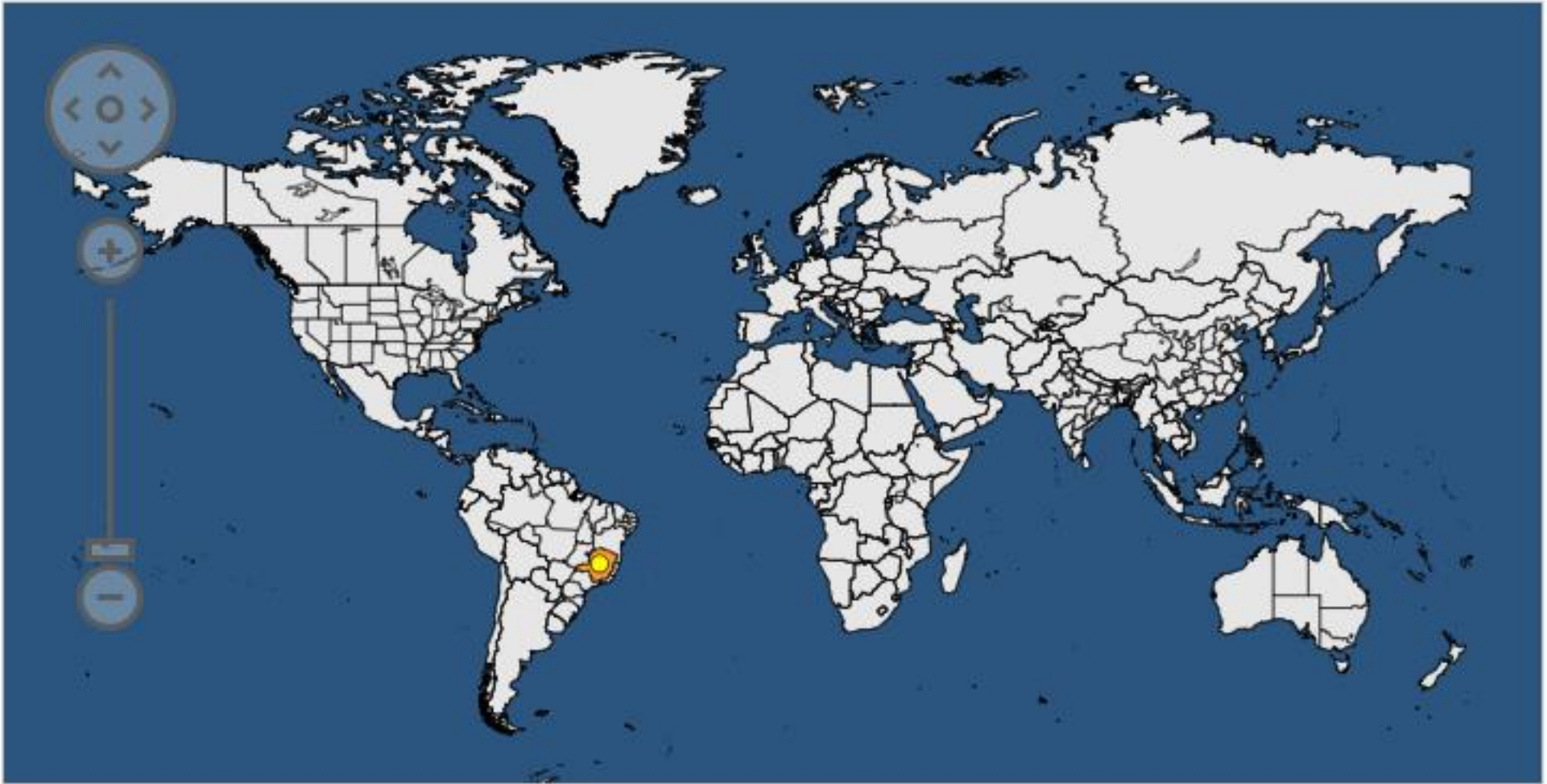
Distribution of *Elsinoe fawcettii*



EPP0. Last updated: 2026-02-13



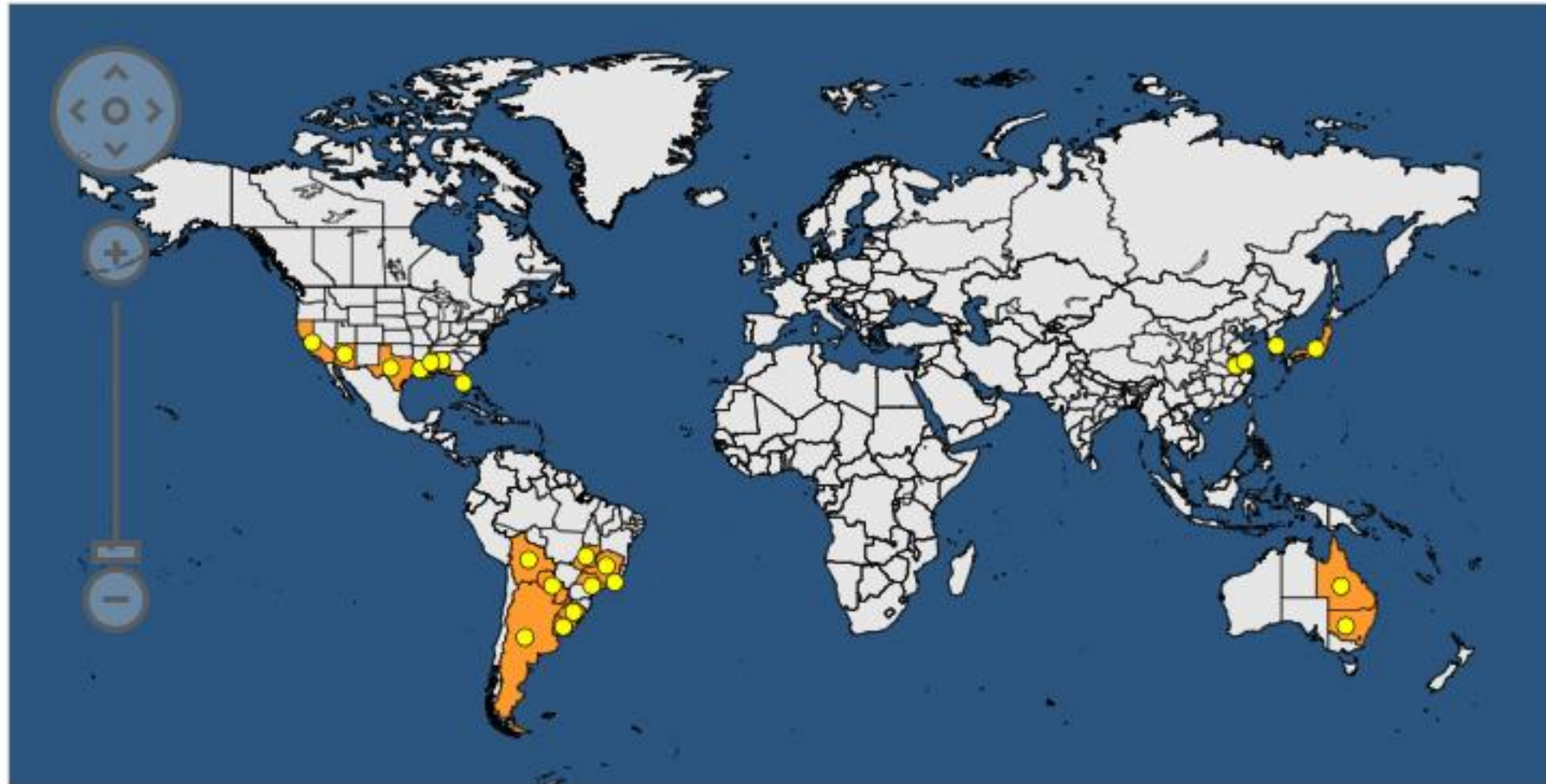
Distribution of *Elsinoe citricola*



EPP0. Last updated: 2024-12-05



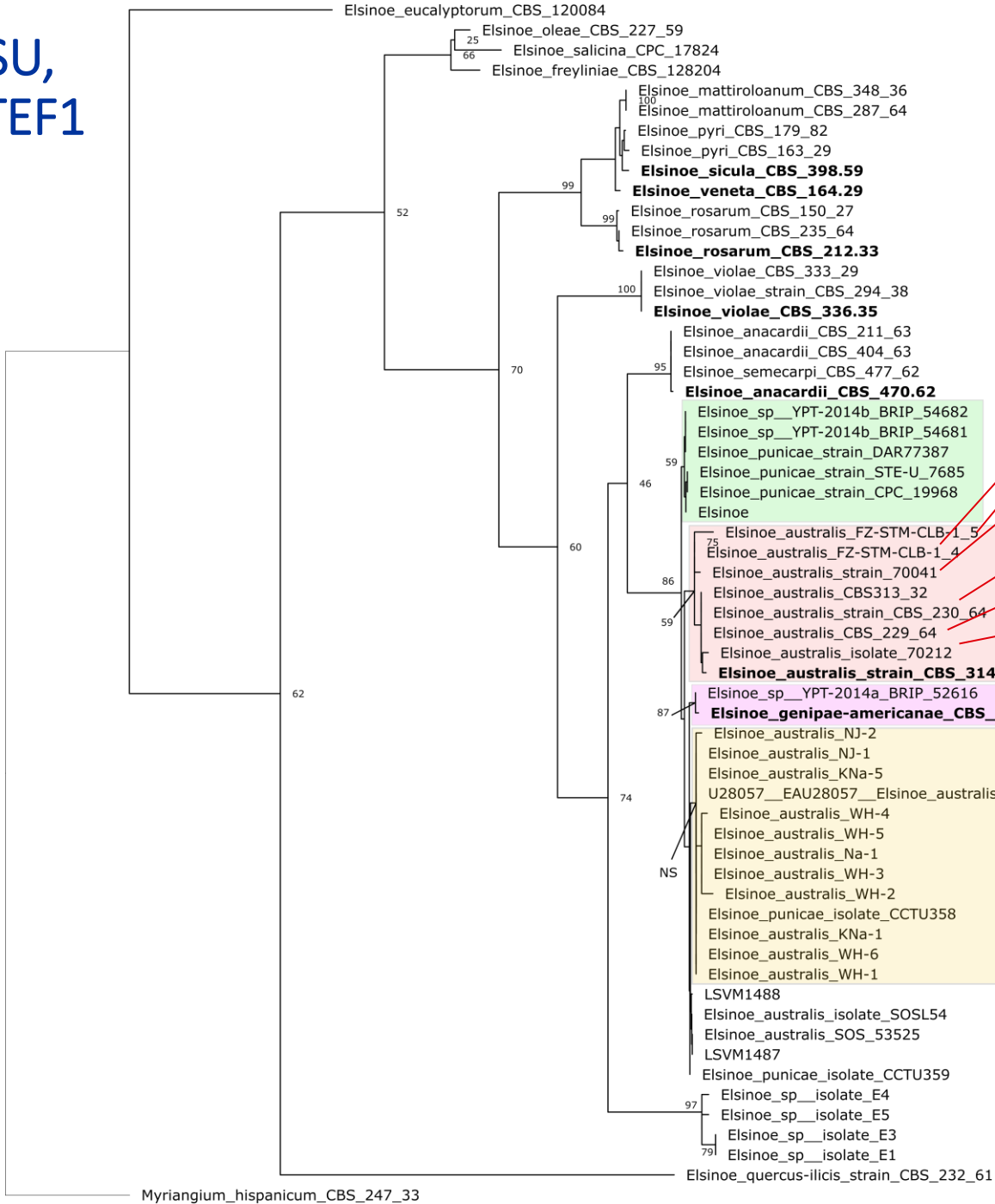
Distribution of *Elsinoe australis*



EPPO. Last updated: 2025-03-17



ITS, LSU, RPB2, TEF1

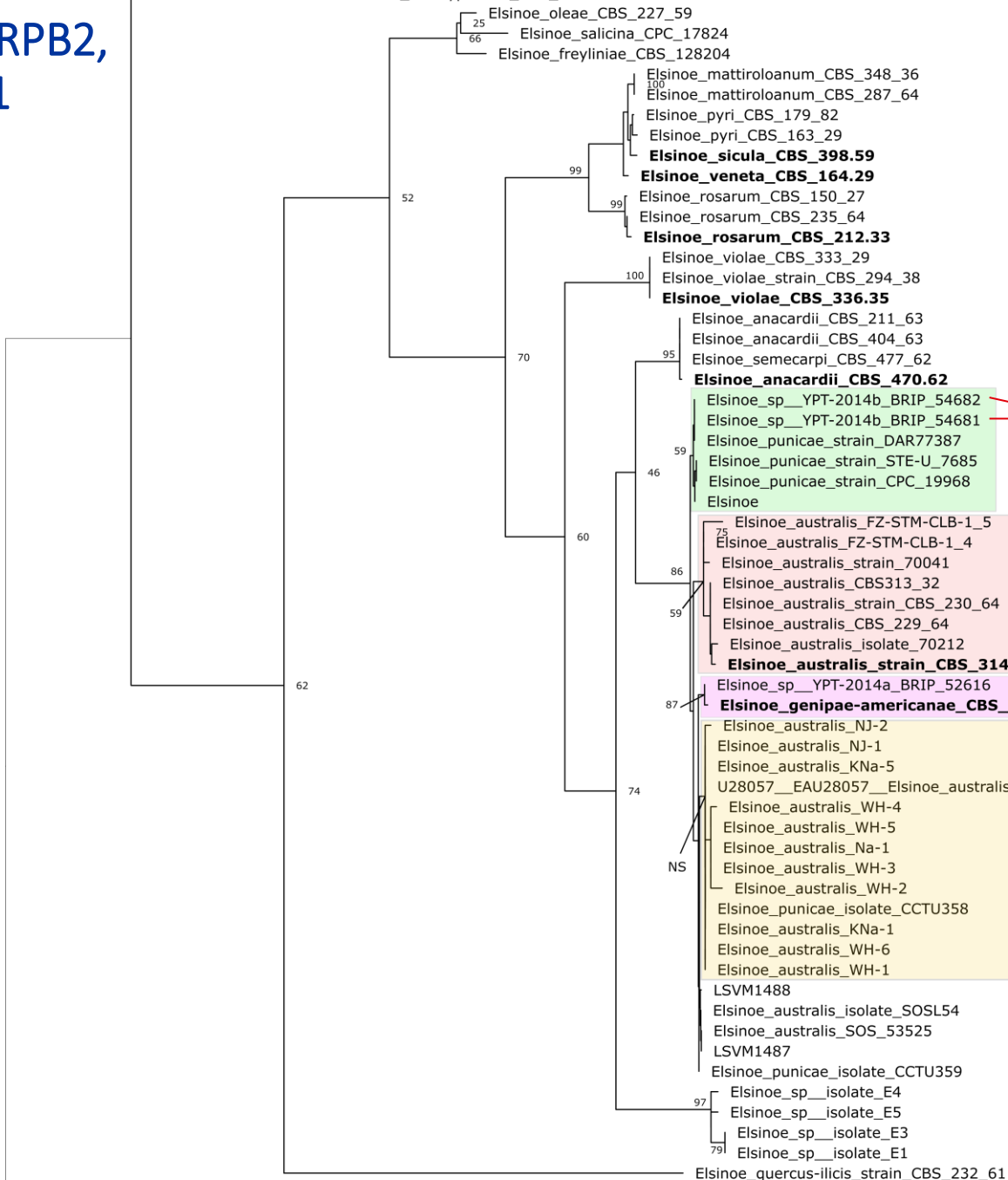


- Sweet orange, Brazil (TEF1)
- Satsuma mandarin, South Korea (ITS, TEF1)
- Citrus aurantium*, Argentina (ITS, LSU, RPB2, TEF1)
- Citrus aurantiifolia*, Brazil (ITS, LSU, RPB2, TEF1)
- Satsuma mandarin, South Korea (ITS, TEF1)
- Citrus aurantium*, Brazil (ITS, LSU, RPB2, TEF1)

Sequences from Fan et al. 2017 and from the NCBI

The best model of evolution was selected for each partition according to ModelTest. Reconstruction was made using RAxML, 1000 pseudoreplicates. Length: 2239 bp.





Novel Pathotypes of *Elsinoë australis* Associated with *Citrus australasica* and *Simmondsia chinensis* in Australia

Andrew K. Miles · Yu Pei Tan · Roger G. Shivas · André Drenth

Isolated from *Simmondsia chinensis*
(Jojoba or deer nut)

Australasian Plant Pathology (2018) 47:405–411
<https://doi.org/10.1007/s13313-018-0572-x>

RESEARCH NOTE



Elsinoë punicae causing scab of pomegranates in South Africa does not cause disease on citrus

Elma Carstens^{1,2} · Shaun D. Langenhoven² · Romain Pierron² · Wilhelm Laubscher³ · Jakobus J. Serfontein⁴ · Carolien M. Bezuidenhout⁵ · Elrita Venter⁶ · Paul H. Fourie^{1,2} · Vaughan Hattingh^{1,7} · Lizel Mostert²



ITS, LSU,
RPB2, TEF1

Novel Pathotypes of *Elsinoë australis* Associated with *Citrus australasica* and *Simmondsia chinensis* in Australia

Andrew K. Miles · Yu Pei Tan · Roger G. Shivas ·
André Drenth

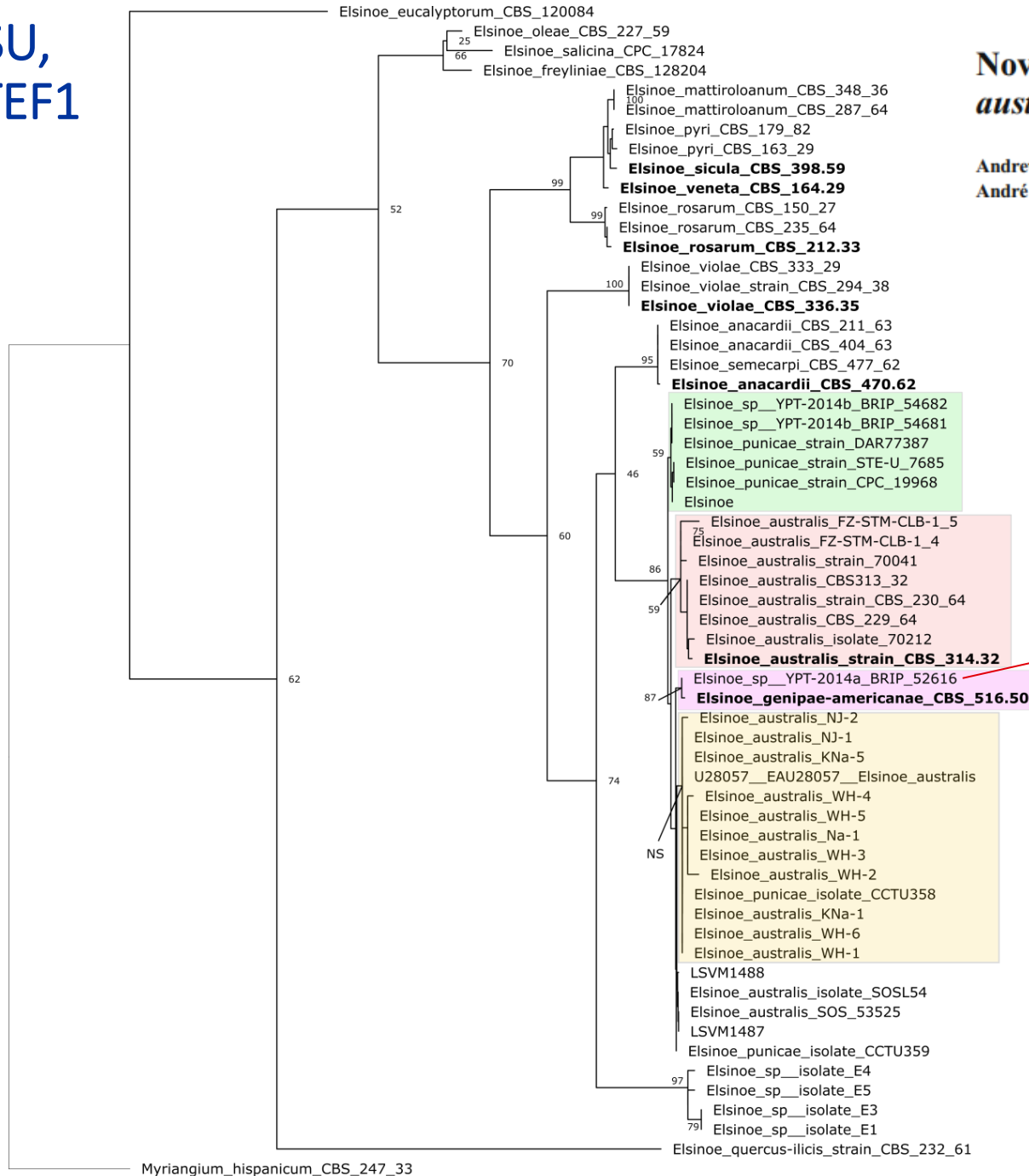


Fig. 1 Fruit of *Citrus australasica* (finger lime) with scab-like lesions. Arrow indicates where tissue was excised for isolation



The best model of evolution was selected for each partition according to ModelTest. Reconstruction was made using RAxML, 1000 pseudoreplicates. Length: 2239 bp.



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Pathotypes and Genetic Relationship of Worldwide Collections of *Elsinoë* spp. Causing Scab Diseases of Citrus

J. W. Hyun, S. H. Yi, S. J. MacKenzie, L. W. Timmer, K. S. Kim, S. K. Kang, H. M. Kwon, and H. C. Lim

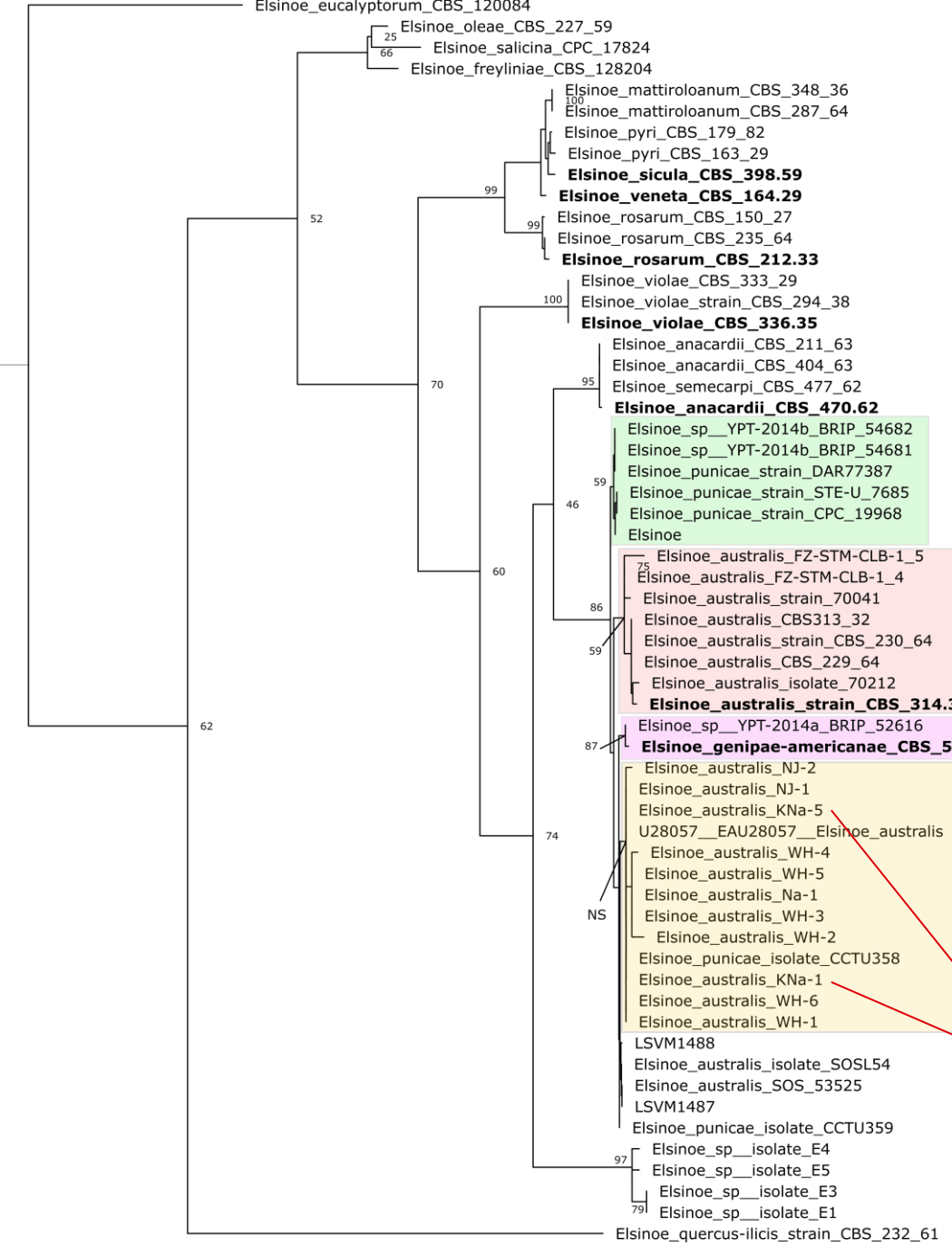
First, fifth, and seventh authors: Citrus Experiment Station, National Institute of Subtropical Agriculture, R.D.A. Jeju, 697-943, S. Korea; second author: National Instrumentation Center for Environmental Management (NICEM), Seoul National University, Seoul, 151-921, S. Korea; third author: University of Florida, Gulf Coast Research and Education Center, Wimauma 33598; fourth author, University of Florida, Citrus Research and Education Center, Lake Alfred 33850; sixth author: Korea National Agricultural College, R.D.A., Kyonggi-do, 445-893, S. Korea; eighth author: Subtropical Environment Division, National Institute of Subtropical Agriculture, R.D.A., Jeju, 690-150, S. Korea.
Accepted for publication 17 February 2009.

TABLE 1. Isolates of *Elsinoë* spp. from citrus worldwide, their host and country of origin, pathogenicity to a range of hosts in the greenhouse tests, pathotype designation, and designations for the sequences of the translation elongation factor α (TEF) and internal transcribed spacer (ITS)

Isolate ^a	Location, no, designation	Host ^b	Pathogenicity ^c							Pathotype ^d	ITS/TEF genotype ^e	
			SM	RL	SO	GF	CM	NDF	JIN			Clem
Korea (Jeju Island)												
1, SM3-1		SM	+	+	+	+	+	-	-	N	FBHR	A-1
2, SM12-1		SM	+	+	±	+	+	-	-	N	FNHR	A-1
3, SM12-2		SM	+	+	+	+	+	-	-	N	FBHR	A-1
4, SM12-5		SM	+	+	+	+	+	N	-	N	FBHR	A-1
5, SM16-1		SM	+	+	+	+	+	N	-	+	FBHR	A-1
6, SM16-3		SM	+	+	+	+	+	N	-	N	FBHR	A-1
7, SM16-4		SM	+	+	+	+	+	N	-	N	FBHR	A-1
8, SM16-8		SM	+	+	±	+	+	-	-	N	FNHR	A-1
9, SM24-1		SM	+	+	+	+	+	+	-	+	FBHR	A-1
10, SM24-4		SM	+	+	+	+	+	N	-	N	FBHR	A-1
11, MUD		SM	+	+	+	+	+	N	-	N	FBHR	A-1
12, Yuzu1-3		YZ	+	+	+	+	+	+	-	±	FBHR	A-1
13, Yuzu2-1		YZ	+	+	+	+	+	N	-	N	FBHR	A-1
14, Yuzu2-2		YZ	+	+	+	+	+	N	-	N	FBHR	A-1
15, Ryuzu-1		YZ	+	+	+	+	+	N	-	±	FBHR	A-1
16, Ryuzu-2		YZ	+	+	+	+	+	N	-	+	FBHR	A-1
17, Ryuzu-3		YZ	+	+	+	+	+	N	-	N	FBHR	A-1
18, Maru-1		KK	+	+	+	+	+	-	N	±	FBHR	A-1
19, Maru-2		KK	+	+	+	+	+	N	N	±	FBHR	A-1
20, Maru-3		KK	+	+	+	+	+	N	N	N	FBHR	A-1
21, Maru-5		KK	+	+	+	+	+	N	N	N	FBHR	A-1
22, Jin-1		JIN	-	+	-	-	-	-	-	+	Jingoul	A-3-2
23, Jin-2		JIN	-	+	-	-	-	±	N	+	Jingoul	A-3-2
24, Jin-3		JIN	-	+	-	-	-	-	-	+	Jingoul	A-3-2
25, Jin-6		JIN	-	+	-	-	-	±	N	+	Jingoul	A-3-1
26, Jin-7		JIN	-	+	-	-	-	±	N	+	Jingoul	A-3-1
27, Jin-8		JIN	-	+	-	-	-	±	N	+	Jingoul	A-3-2
28, Na-1		NDF	-	-	-	-	-	+	-	-	Natsudaikai	B-1-3
29, Na-2		NDF	-	-	-	-	-	+	-	N	Natsudaikai	B-1-3
30, KNa-1		NDF	-	-	-	-	-	+	-	-	Natsudaikai	B-1-2
31, KNa-2		NDF	-	-	-	-	-	+	N	N	Natsudaikai	B-1-2
32, KNa-5		NDF	-	-	-	-	-	+	N	N	Natsudaikai	B-1-1
33, KNa-9		NDF	-	-	-	-	-	+	N	N	Natsudaikai	B-1-2

(continued on next page)

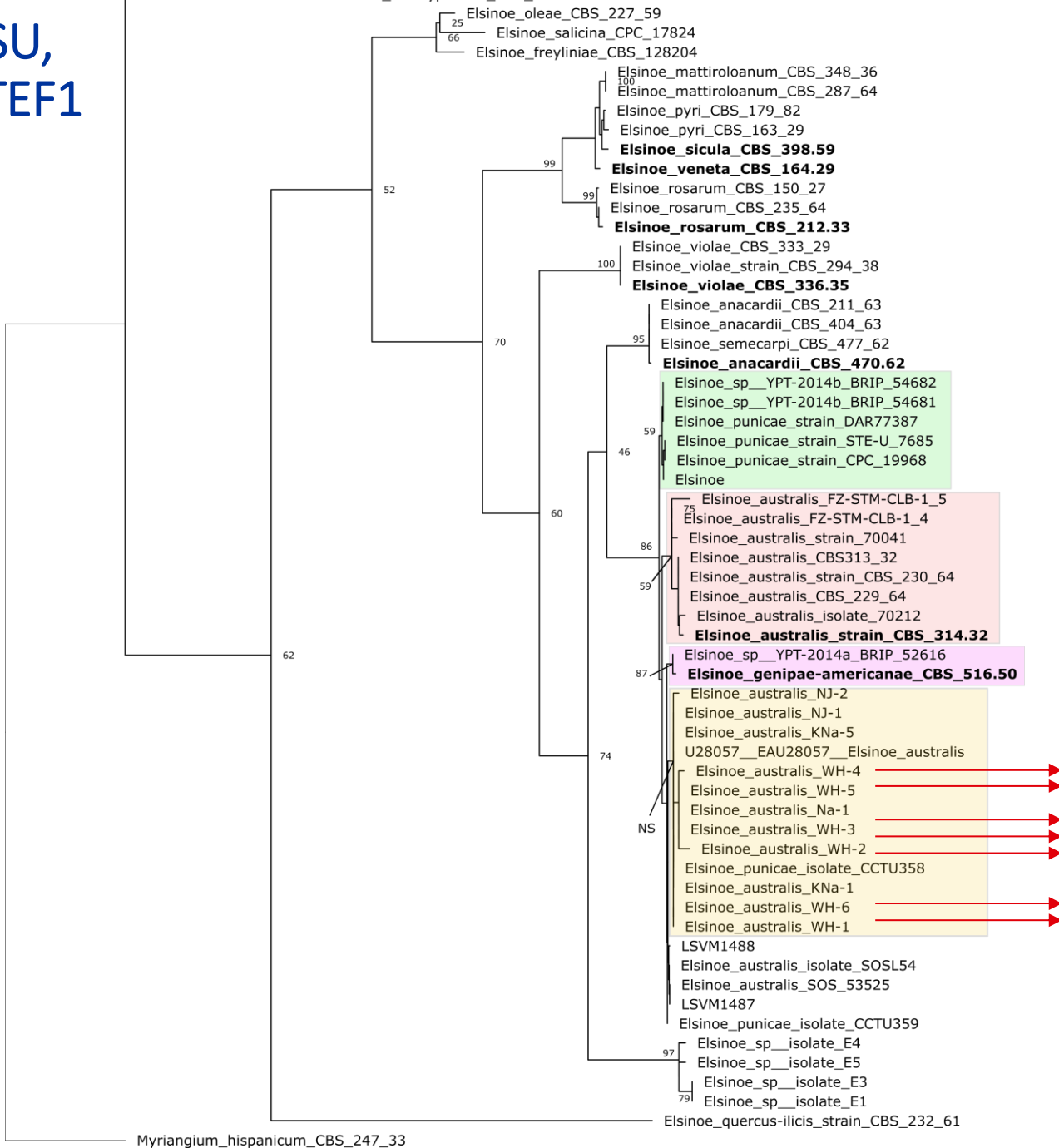
Kna-1, Kna-5 pathogenic on natsudaikai fruit
(Citrus natsudaikai)



Myriangium_hispanicum_CBS_247_33

ITS, LSU, RPB2, TEF1

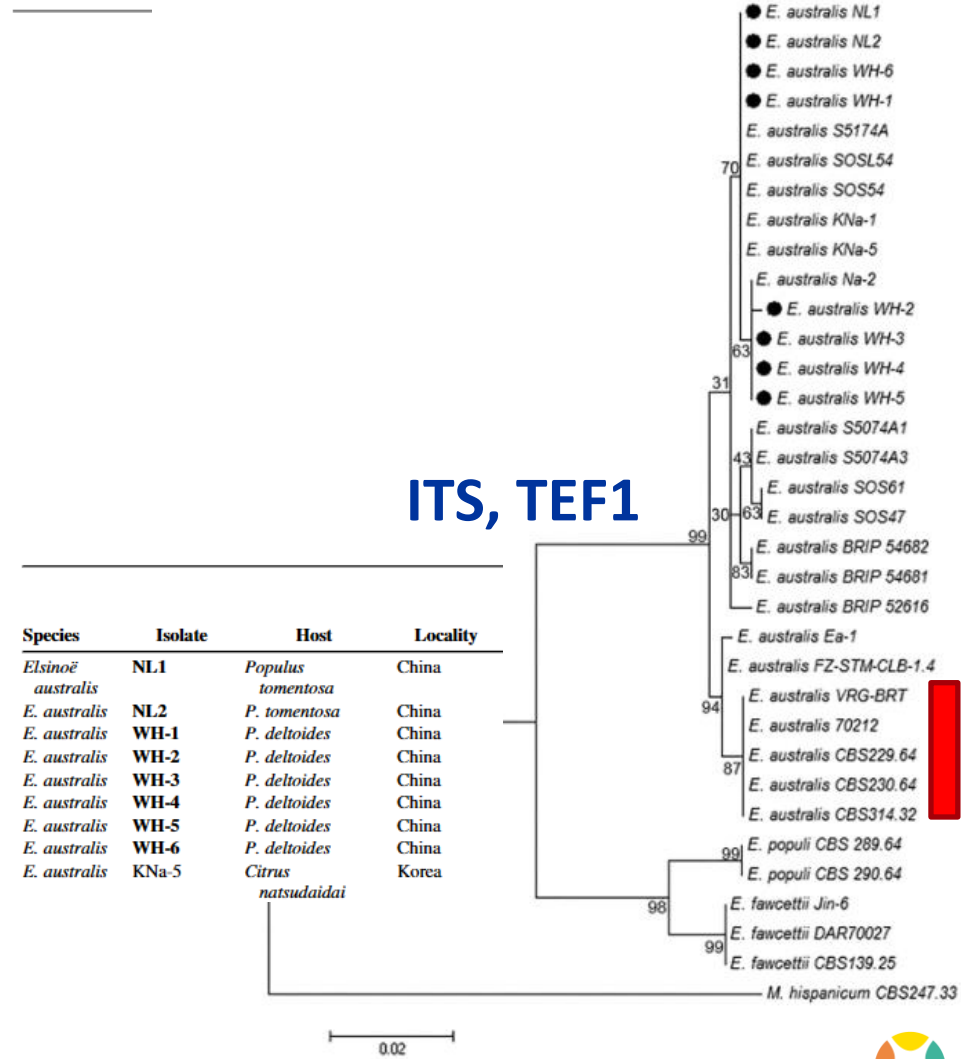
Elsinoe_eucalyptorum_CBS_120084



Elsinoë australis Causing Spot Anthracnose on Poplar in China

Lijuan Zhao, Hongju Xiao, Xujie Ma, and Qiang Cheng[†]

Key Laboratory of Forest Genetics & Biotechnology of Ministry of Education, Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China



Species	Isolate	Host	Locality
<i>Elsinoë australis</i>	NL1	<i>Populus tomentosa</i>	China
<i>E. australis</i>	NL2	<i>P. tomentosa</i>	China
<i>E. australis</i>	WH-1	<i>P. deltoides</i>	China
<i>E. australis</i>	WH-2	<i>P. deltoides</i>	China
<i>E. australis</i>	WH-3	<i>P. deltoides</i>	China
<i>E. australis</i>	WH-4	<i>P. deltoides</i>	China
<i>E. australis</i>	WH-5	<i>P. deltoides</i>	China
<i>E. australis</i>	WH-6	<i>P. deltoides</i>	China
<i>E. australis</i>	KNa-5	<i>Citrus natsudaidai</i>	Korea

ITS, TEF1

0.02



Presence of non-regulated *Elsinoe* species on symptomatic fruits

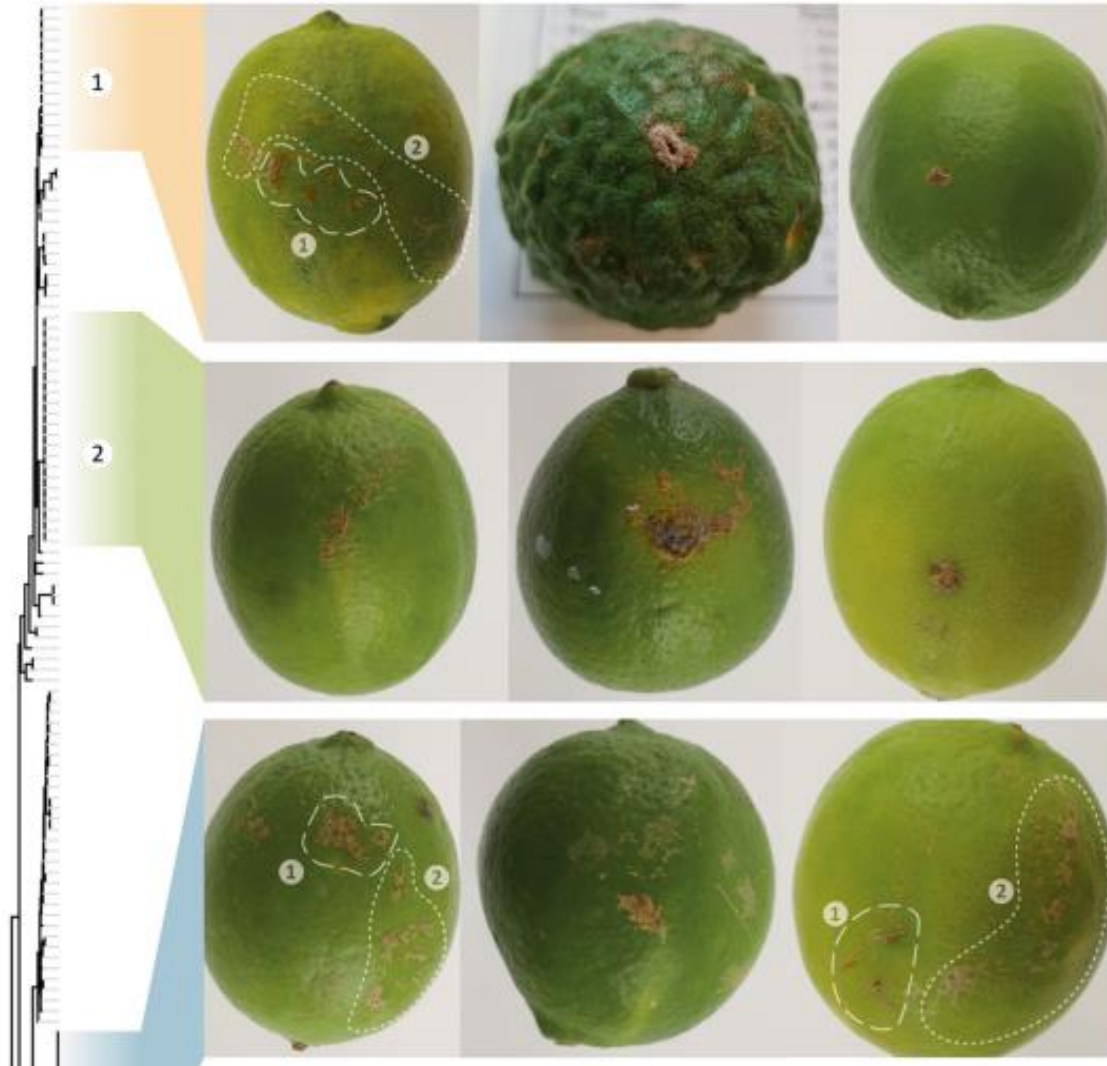


Image credits: **Van de Vossenberget al. 2025**. Presence of non-regulated *Elsinoë* species on citrus fruits and their impact on regulatory plant health diagnostics. *European Journal of Plant Pathology*.

Metabarcoding study

Presence of non-regulated *Elsinoë* species on citrus fruits

- ***Elsinoë* species** other than the EU-regulated are present in citrus fruits (metabarcoding).

Life strategy:

- Pathogenic?
- Endophytic?
- Saprophytic?

- The new findings have implications on the development of new detection methods.

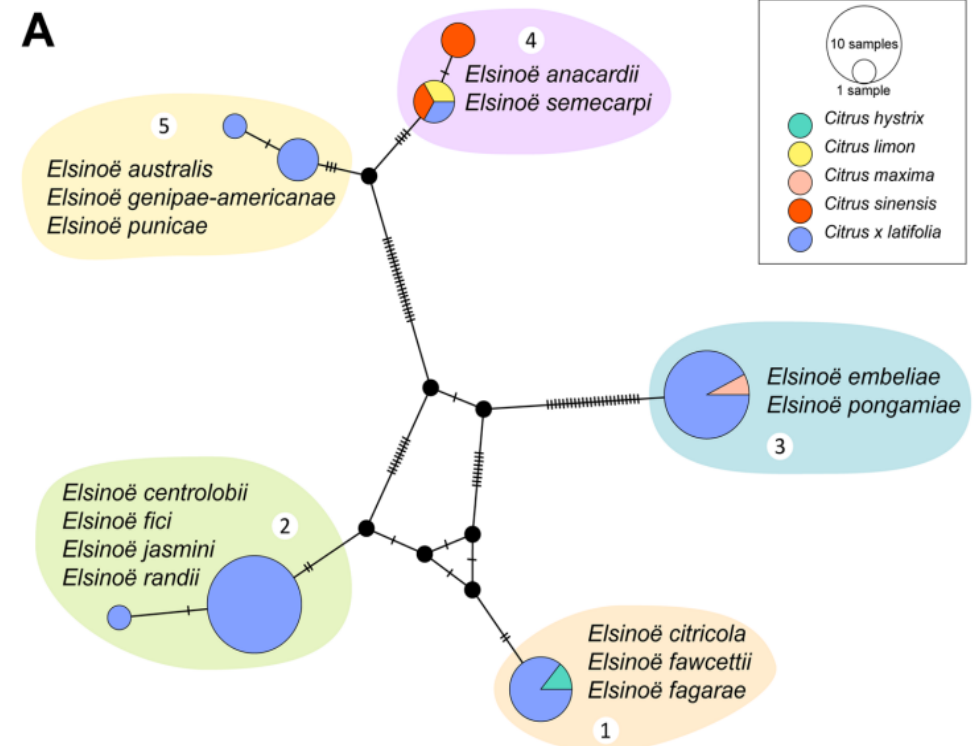
Eur J Plant Pathol
<https://doi.org/10.1007/s10658-025-03168-0>



Presence of non-regulated *Elsinoë* species on citrus fruits and their impact on regulatory plant health diagnostics

Bart T. L. H. van de Vossenbergh · Valerie A. van Ingen-Buijs · Ashleigh J. Elliott · Tijs J. M. van den Bosch · Mandy M. D. A. Wildhagen · Aron A. L. A. M. van Duijnhoven · Sietse van der Linde

Fig. 2 Integer Neighbour-Joining network containing 46 *Elsinoë* spp. ITS1 sequences obtained from symptomatic citrus fruit consignments. Circle sizes are proportional to haplotype frequencies, and circles are coloured based on host (A) or consignment origin. (B) Black nodes represent hypothetical ancestors and marks on the branches indicate the number of mutations



Presence of non-regulated *Elsinoe* species on citrus fruits

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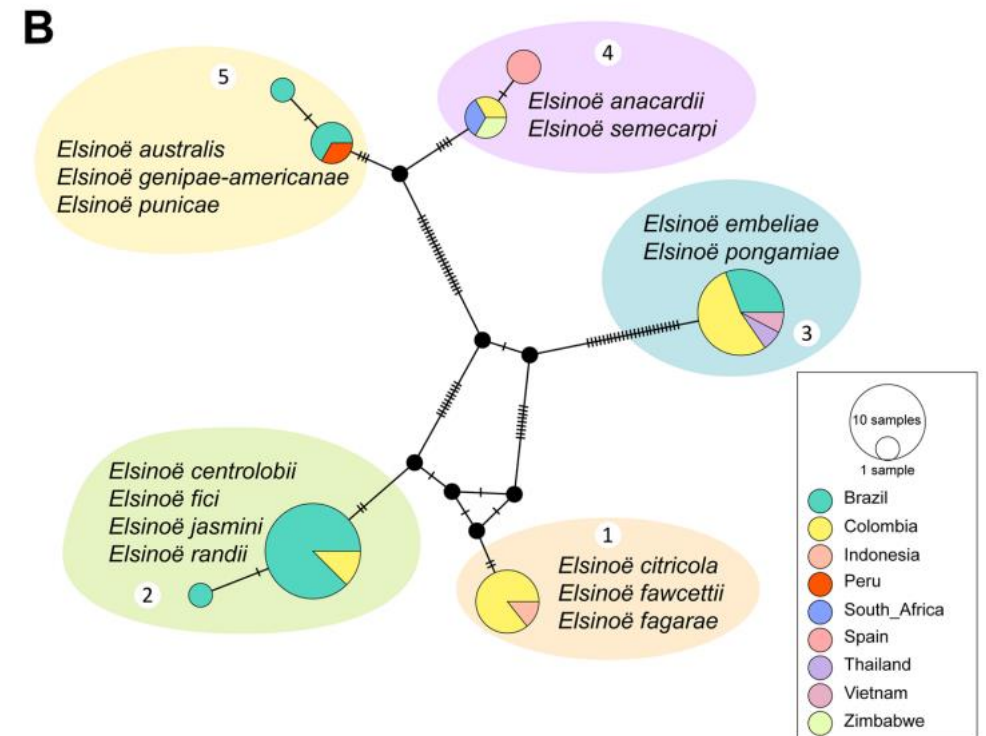
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This new scenario required a broader approach



- **More genomic data** included in the analysis for the selection of candidate genes and design of primers and probes (**TaqMan chemistry**).
- **Higher number** of strains of EU-regulated species (*E. australis*, *E. citricola*, *E. fawcettii*).
- Additional efforts to **isolate** *Elsinoe* species from scab-symptomatic (citrus) **fruits**.
- Extended assessment of the **specificity** by including more *Elsinoe* species.

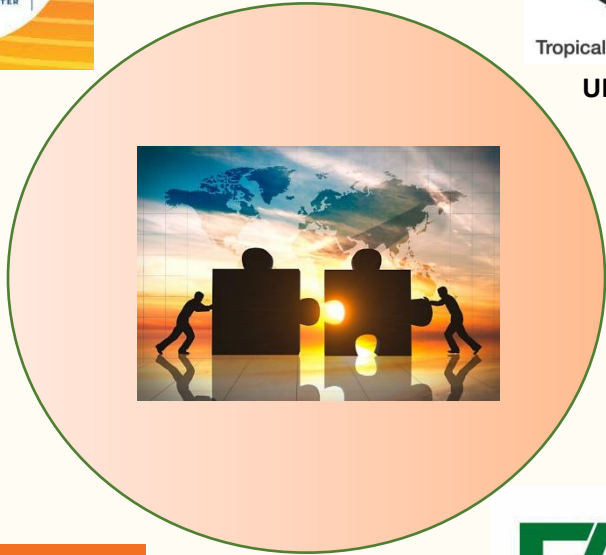


Credits: Wikipedia

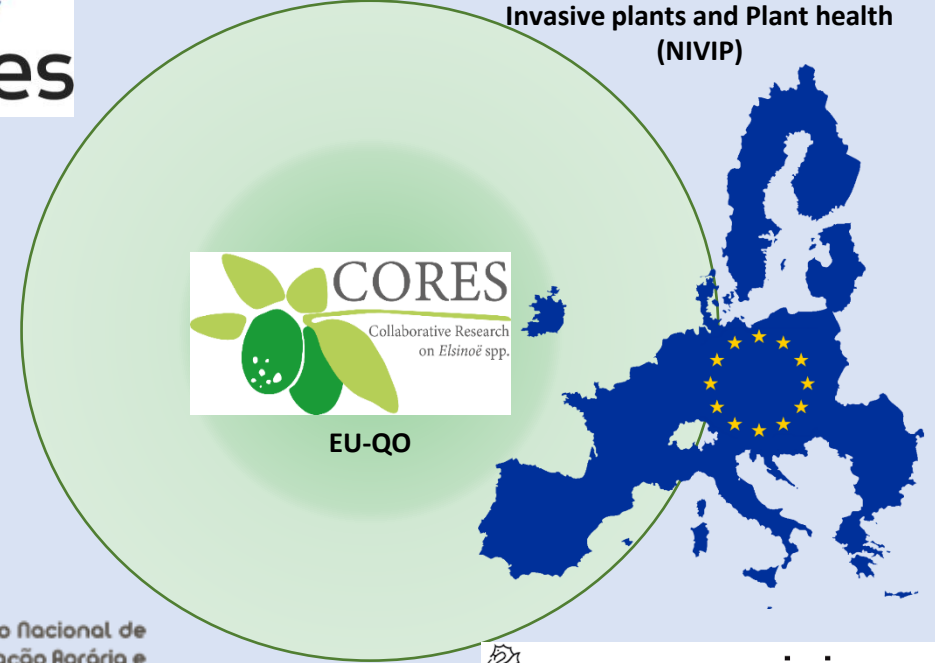
Multilateral and multidisciplinary research



Tropical Plant Pathology
UF/IFAS PDC



Netherlands Institute for Vectors,
Invasive plants and Plant health
(NIVIP)



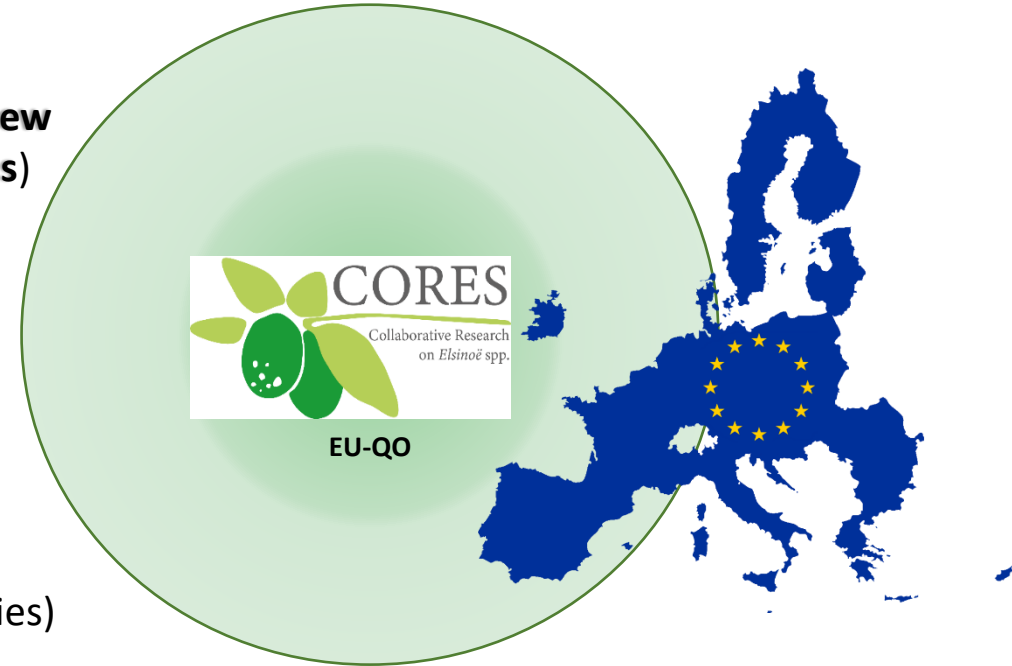
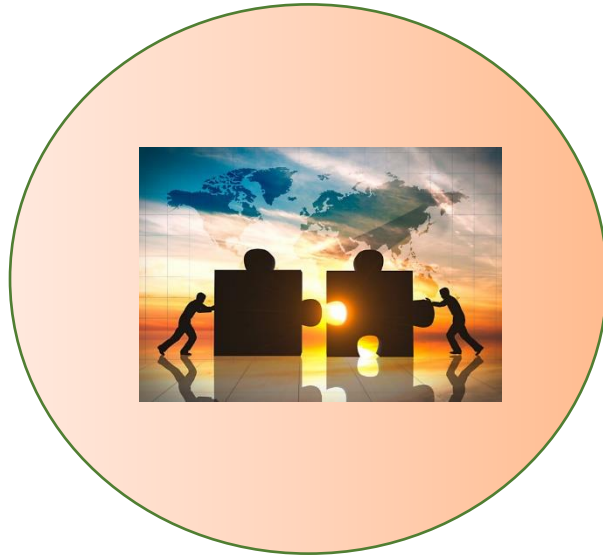
Multilateral and multidisciplinary research

Over **20 strains** of *Elsinoe* isolated from several Citrus species (yet to be fully characterized)

Genomes for >50 *Elsinoe* species

gDNA from ~63 **non-regulated *Elsinoe* species** (exclusivity)

Elsinoe sp. Isolated from Citrus (**new distribution and host associations**)



Living strains of EU-regulated *Elsinoe* species (inclusivity studies)

In **field collection** of Scab-symptomatic fruits (not only citrus)

>10 strains of *Elsinoe* species isolated from **tropical/subtropical fruit trees**

Local surveys

gDNA extracted from symptomatic fruits (**consignments**)

Cooperation



Expertise
More Biological Material



More reliable detection assays

Key Takeaways

- Multilocus Sequence Analysis (**MLSA**) is strongly suggested rather than single-locus or two-loci phylogeny for a correct placement of *Elsinoe* isolates.
- These findings highlight the importance of **accurate identification** and **understanding** of the mycoflora associated to the pathosystem before detection methods are developed (**primers and probes**)
- **Partnership** with other EU-NRLs and international institutions has greatly contributed to our understanding of EU-regulated *Elsinoe* species (and other non-regulated species)
- **Genomic data** from *Elsinoe* species that are closely related to the EU-regulated *Elsinoe* species has been crucial for the development of highly specific detection assays
- The EURL has now real time PCR assays targeting *E. australis* and *E. fawcettii*. i.e.; **no cross amplification with non-regulated *Elsinoe* species** (34 species assessed so far).
- **Ongoing**: Selection of new candidate regions to design primers/probes targeting *E. citricola* (specificity).





THANK YOU



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